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(71) Applicant: REPLIGEN CORPORATION [US/US]; One Kendall Square, Building 700, Cambridge, MA 02139 (US).

(72) Inventors: HIGGINS, Paul, J. ; 8 Sheridan Avenue, Medford, MA 02155 (US). POTTS, Barbara, J. ; 37 Grant Street, Lexington, MA 02173 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).

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(54) Title: HETEROCONJUGATE ANTIBODIES FOR TREATMENT OF HIV INFECTION

(57) Abstract

The invention features a heteroconjugate antibody which includes two binding functionalities. The first binding function is directed to a peripheral blood effector cell antigen, preferably CD-3. The second functionality is directed to a specific domain of an aids virus coat protein, preferably the V3 loop sequence of the gp120 envelope from HIV MN or a variant thereof. Methods of therapy are also presented where 20 ng/ml of a mixed culture of HIV infected cells (effector/CEM-ss cells) have a 80-90 % decrease in reverse transcriptase activity compared to an identical culture with a 3:1 ratio of effector cell:CEM-ss.

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HETEROCONJUGATE ANTIBODIES FOR TREATMENT OF HIV INFECTION

Background of the Invention

This invention relates to the treatment of Human Immunodeficiency Virus infection.

Human Immunodeficiency Virus (HIV), the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS), is a retrovirus which infects certain immune system cells, including T4 lymphocytes and $CD4^+$ cells of the monocyte/macrophage lineage. In the absence of effective treatment, the mortality rate for AIDS patients approaches 100% (Fauci, *Science* 239:617, 1988).

Well over 100 HIV variants have been identified. The amino acid sequence of the HIV envelope glycoprotein gp120 is particularly variable; its amino acid sequence can vary by 20-25% from one strain to the next. In addition to strain to strain variability, there is a more subtle variation in genome sequence caused by the high error rate of reverse transcriptase. The misincorporation rate is high enough to introduce one error per genome per replication cycle. Consequently any particular viral isolate consists of a cohort of quasi-species. Further, the diversity and number of quasi-species apparently differs from one HIV variant to another. There is substantial evidence that these quasi-species evolve *in vivo*. For example, successive viral isolates from an infected individual reveal substantial temporal fluctuations in the proportion of various quasi-species (Meyehans, *Cell* 58:901, 1989). There is also evidence that neutralization-resistant HIV variants can arise through single-base changes in the viral sequence encoding gp120 when HIV is grown in the presence of neutralizing antibodies (Reitz et al., *Cell* 54:57, 1988). Infected individuals initially mount a humoral and

cellular immune response against HIV, and there is reason to believe that an infected individual's immune response may actually encourage viral spread and the emergence of more resistant variants (McCune et al., *Cell* 64:351, 1991).

Human monoclonal antibodies directed against HIV proteins have been produced by hybridoma formation and Epstein Barr Virus transformation (Banapour et al., *J. Immunol.* 139:4027, 1987; Amadoci et al., *AIDS Res. and Human Retroviruses* 5:73, 1989).

More recently cytotoxic hybrid proteins composed of a cytotoxin fused to part of the CD4 receptor have been proposed as a way to destroy cells expressing HIV encoded proteins. This approach relies on the fact that the HIV envelope protein, gp120, recognizes the CD4 receptor, which is present on T4 lymphocytes and certain cells of the monocyte/macrophage lineage. Thus, a soluble derivative of CD4 might be used to target a cytotoxin to HIV infected cells that express surface gp120. Chaudhary et al. (*Nature* 335:369, 1988) found that administration of a CD4-*Pseudomonas* exotoxin hybrid protein to a lymphocytic cell line chronically infected with HIV causes a decrease in overall protein synthesis. Till et al. (*Science* 242:1166, 1988) found that a CD4-ricin A fusion protein decreases DNA synthesis in cultures of chronically infected H9 cells. In a variation of this strategy, Capon et al. (*Nature* 337:529, 1989) designed a hybrid protein composed of soluble CD4 and the constant region of an antibody. This molecule is designed to direct immune system response to gp120. Another molecule of this general type has been shown to activate complement (Traunecker et al. *Nature* 339:78, 1989).

Heteroconjugate molecules consisting of two covalently joined antibodies or an antibody covalently

joined to a cell- or virus-targeted protein have been proposed as a means by which to target cytotoxic cells to undesirable cells such as tumor cells and virally infected cells. Segal et al. (U.S. Patent No. 4,676,980) suggest the use of cross-linked hetero-antibodies to target immune system cells to unwanted or detrimental cells. Fanger et al. (PCT publication WO91/00360) have proposed such heteroconjugates for treatment of AIDS. In particular, Fanger et al. suggest the use of a high affinity Fcγ receptor-specific antibody fused to CD4 (or the CD4 binding domain of gp120) for AIDS therapy. Fanger et al. also suggest the use of heteroantibodies consisting of an high affinity Fcγ receptor-specific antibody fused to an HIV-specific antibody such as anti-gp120 antibody for AIDS therapy. Zarling et al. (EP Publication No. 03089.36) described heteroconjugate antibodies consisting of an antibody specific for an HIV antigen that is expressed on HIV infected cells cross-linked to a second antibody which is specific for an effector cell of the peripheral blood and which is capable of killing HIV infected cells.

Summary of the Invention

In general, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein the heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN

decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In a preferred embodiment, the decrease in the reverse transcriptase activity of the first cell culture is greater than 90% compared to the reverse transcriptase activity of the second mixed cell culture.

In another preferred embodiment, the heteroconjugate antibody at an initial concentration of 200 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with an HIV strain other than HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 50% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and said CEM-ss cells infected with the HIV strain other than HIV-MN, wherein the effector cells are in 3-fold excess over said CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to said CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-

1000 infectious units of the HIV strain other than HIV-MN.

In yet another preferred embodiment, the heteroconjugate antibody binds to the V3 loop of an HIV strain other than HIV-MN.

In other preferred embodiments, the effector cell is chosen from the group consisting of cytotoxic T lymphocytes, neutrophils, monocytes/macrophages, and large granular lymphocytes; and the antigen present on the surface of an effector cell is CD3.

In a another preferred embodiment, the heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-III_B decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-III_B, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-III_B.

In yet another preferred embodiment, the heteroconjugate antibody at an initial concentration of 20 ng/ml in three or more mixed cell cultures each of which includes effector cells and CEM-ss cells infected with one of the HIV strains: Alabama, Duke 6587-5, Duke 6587-7, Duke 7887-7, SF2, WMJ2, and IIIB, decreases the reverse transcriptase activity of each of the mixed cell culture by 80% compared to the reverse transcriptase

activity of an otherwise identical mixed cell culture which includes effector cells and CEM-ss cells infected with the same strain of HIV, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of the strain of HIV.

In a related aspect, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein the heteroconjugate antibody at an initial concentration of 10 ng/ml in a first mixed cell culture which includes the effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes the effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and

second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In a related aspect, the invention feature a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein the heteroconjugate antibody at an initial concentration of 5 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In another related aspect, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant

expressed on the surface of HIV-infected cells, wherein the heteroconjugate antibody at an initial concentration of 1 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In a related aspect, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against the amino acid sequence GPGRAF.

In a preferred embodiment, the heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase

activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In a related aspect, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against the amino acid sequence IXIGPGR, wherein X = any amino acid.

In a preferred embodiment, the heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In a related aspect, the invention features a heteroconjugate antibody which includes a first and a second portion joined together covalently, the first portion includes an antibody directed against an antigen

present on the surface of an effector cell of the peripheral blood, the second antibody portion includes an antibody directed against the amino acid sequence QARILAVERYLKDQQLLGIWGCSGKLIC.

In a preferred embodiment, the heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture which includes effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of the first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture which includes effector cells and the CEM-ss cells infected with HIV-MN, wherein the effector cells are in 3-fold excess over the CEM-ss cells in the first and second mixed cell cultures, the reverse transcriptase activity is measured ten days after infection, the heteroconjugate antibody and the effector cells are added to the CEM-ss cells in the first mixed cell culture 18 hours after infection, and the first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

In other preferred embodiments, the effector cell is chosen from the group consisting of cytotoxic T lymphocytes, neutrophils, monocytes/macrophages, and large granular lymphocytes; and the antigen present on the surface of an effector cell is CD3.

In another aspect, the invention features a pharmaceutically acceptable composition which includes a pharmaceutically effective amount of a heteroconjugate antibody described above.

In a related aspect, the invention features a method for treating a patient infected with HIV, the method includes administering to the patient the above-described pharmaceutically acceptable composition.

In another aspect, the invention features an HIV-targeted effector cell which includes: (a) an effector cell expressing a cell surface antigen; and (b) an above-described heteroconjugate antibody.

In a related aspect, the invention features a method for treating a patient infected with HIV; the method includes administering to the patient the above-described HIV-targeted effector cell.

The MN prototype virus is defined by a particular amino acid subsequence within the V3 loop region of the gp120 envelope protein having positions A_1-A_{17}:K-R-K-R-I-H-I-G-P-G-R-A-F-Y-T-T-K. (Amino acid sequences are presented in the standard single-letter code throughout.)

MN viral variants are variant which exhibit complete amino acid sequence homology at residues I-G-P-G-R, i.e., at positions A_7 through A_{11}, and at least 36% homology with the remaining 12 amino acids of the HIV-MN sequence given above.

By "directed against" is meant that an antibody binds to the indicated antigen. The V3 loop of gp120 is defined as the 36 amino acid region from amino acid 303 to 338, inclusive, according to the gp120 numbering scheme of Ratner et al. (*Nature* 313:277, 1985).

The heteroconjugate antibodies of the invention are highly effective; even at low concentrations they are capable of nearly eliminating viral replication as judged by a reverse transcriptase assay. The preferred heteroconjugate antibodies are those which are effective against more than one strain.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first briefly described.

Figure 1 is a graphical representation of the effect of a mixture of unconjugated OKT3 antibody and 59.1 antibody (filled diamonds) and OKT3/59.1 heteroconjugate antibody (open squares) on the reverse transcriptase activity of CEM-ss cells infected with HIV-III_B in the presence of cytotoxic T-lymphocytes. Reverse transcriptase activity (cpm/10 μl) is presented as a function of the initial antibody concentration (ng/ml) in the cell culture.

Figure 2 is a graphical representation of the effect of a mixture of unconjugated OKT3 antibody and 59.1 antibody (filled diamonds) and OKT3/59.1 heteroconjugate antibody (open squares) on the reverse transcriptase activity of CEM-ss cells infected with HIV-MN in the presence of cytotoxic T-lymphocytes. Reverse transcriptase activity (cpm/10 μl) is presented as a function of the initial antibody concentration (ng/ml) in the cell culture.

Figure 3 is a graphical representation of the effect of a mixture of unconjugated OKT3 antibody and 59.1 antibody (open circles) and OKT3/59.1 heteroconjugate antibody (filled circles) on the reverse transcriptase activity of CEM-ss cells infected with HIV-III_B in the absence of cytotoxic T-lymphocytes. The reverse transcriptase activity of HIV-III_B infected CEM-ss cells in the presence of cytotoxic lymphocytes only (filled triangle); HIV-III_B infected CEM-ss cells alone (open triangle); and uninfected CEM-ss cells alone (filled square) is also indicated. Reverse transcriptase activity (cpm/10 μl) is presented as a function of the initial antibody concentration (ng/ml) in the cell culture (except for those cases in which no antibody was added).

Figure 4 is a graphical representation of the effect of the ratio of cytotoxic T-lymphocytes to HIV-MN

infected CEM-ss cells on reverse transcriptase activity in the presence of 1 μg/ml OKT3/59.1 heteroconjugate antibody. Reverse transcriptase activity (cpm/10 μl) is presented as a function of the cytotoxic lymphocyte to CEM-ss cells (log_{10} scale).

Figure 5 is a set of graphs which illustrate the effect of cytotoxic T-lymphocytes and OKT3/59.1 heteroconjugate antibody (open squares) and a mixture of unconjugated OKT3 antibody and 59.1 antibody (filled triangles) on the reverse transcriptase activity of CEM-ss cells infected with HIV-MN (panel A), HIV-Alabama (panel B), HIV-Duke 7887-7 (panel C), HIV-Duke 6587-5 (panel D), HIV-Duke 6587-7 (panel E), $HIV\text{-}III_B$ (panel F), HIV-SF2 (panel G), and HIV-WMJ2 (panel H). In each case, a control is included where neither antibodies or cytotoxic T-lymphocytes were added (filled circles). Reverse transcriptase activity (cpm/10 μl) is presented as a function of the number of days post-infection.

Figure 6 is a graphical representation of the effect of a mixture of conjugated OKT3 antibody and 6C5 antibody (open circles) and OKT3/6C5 heteroconjugate antibody (filled circles) on the reverse transcriptase activity of $HIV\text{-}III_B$ infected CEM-ss cells. Reverse transcriptase activity (cpm/10 μl) is presented as a function of the initial antibody concentration (ng/ml) in the cell culture.

Heteroconjugate Antibodies for AIDS Therapy

The molecules of the invention are heteroconjugate antibodies produced by covalently attaching a first antibody which is directed against an antigen present on the surface of a cytotoxic immune effector cell capable of killing an HIV infected cell to a second antibody which is directed against an HIV antigen present on the surface of HIV infected cells.

The heteroconjugate antibodies of the invention are highly potent. Even at relatively low concentrations, these heteroconjugate antibodies are capable of substantially reducing HIV activity in a mixed cell culture of HIV infected cells and effector cells. The most preferred heteroconjugate antibodies are those which are both highly potent and broadly reactive. Broadly reactive heteroconjugate antibodies are those which are effective against more than one strain of HIV. For example, a broadly reactive heteroconjugate antibody might be effective against HIV-MN and HIV-SF2 or HIV-MN and HIV-WMJ2, or HIV-MN and $HIV\text{-}III_B$.

The portion of the heteroconjugate antibody which is directed against a cytotoxic immune effector cell capable of killing HIV infected cells recognizes an antigen present on the surface of cells such as: cytotoxic T-lymphocytes, monocytes/macrophages, large granular lymphocytes (including cells and NK cells), and neutrophils. Preferably, the immune effector cell-directed antibody binds to an antigen on the surface of the effector cell in a manner which triggers cytolytic activity. For example, the antigen recognized can be the CD3 receptor or the CD16 (Fc) receptor. Less preferred are antibodies directed to receptors which require multiple signals to initiate cytolytic activity (e.g., the CD2 and CD28 receptors).

The portion of the heteroconjugate antibody which is directed against an antigen present on the surface of HIV infected cells preferably recognizes: (1) an epitope within the V3 loop sequence of the gp120 envelope protein of the MN prototype of HIV-1 (HIV-MN); (2) an epitope within the V3 loop sequence of the gp120 envelope protein of a viral variant of the MN prototype of HIV-1; or (3) an epitope within the portion of gp41 between amino acids 584 to 611, inclusive.

The V3 loop of gp120 is the 36 amino acid region from amino acid 303 to 338, inclusive, according to the gp120 numbering scheme of Ratner et al. (*Nature* 313:277, 1985). The MN prototype of HIV-1 is defined by the following amino acid subsequence within the V3 loop of gp120: K-R-K-R-I-H-I-G-P-G-R-A-F-Y-T-T-K (A^1-A^{17}). MN viral variants are variants which exhibit complete amino acid homology at residues I-G-P-G-R, i.e., positions A^7 through A^{11}, and at least 36% homology with the remaining 12 amino acids of the MN sequence given above.

The above-described HIV-directed antibodies are good candidates for use in generating heteroconjugate antibodies which are highly potent. In some cases, however, the heteroconjugates formed will not be highly potent. Ultimately the usefulness of a particular HIV-directed antibody for production of a heteroconjugate antibody of the invention can only be assessed by producing a heteroconjugate antibody, for example by covalently linking the HIV-directed antibody to an anti-CD3 antibody, and measuring the potency of the heteroconjugate antibody in an appropriate assay. Once a particular HIV-directed antibody has been shown to be useful for generating a potent heteroconjugate antibody, it can be used to generate other heteroconjugate antibodies by covalently linking it to other effector cell antigen-directed antibodies.

As discussed above, the most preferred heteroconjugate antibodies are those which are broadly reactive as well as highly potent. HIV-directed antibodies which recognize: (1) an epitope having the sequence G-P-G-R-A-F; (2) an epitope having the sequence I-X-I-G-P-G-R, where X is any amino acid; or (3) an epitope within the portion of gp41 between amino acids 584 to 611 (according to the numbering scheme of Ratner et al., *supra*), inclusive are likely to be useful for

generating highly potent and broadly reactive heteroconjugate antibodies. This does not imply that antibodies recognizing other epitopes within the V3 loop of HIV-MN, the V3 loop of an HIV-MN viral variant, or gp41 cannot be used to produce a highly potent, broadly reactive heteroconjugate antibodies.

Described below are techniques for generating and screening HIV-directed antibodies useful for preparation of heteroconjugate antibodies, methods for the preparation of heteroconjugate antibodies, and methods for assessing the potency and breadth of reactivity of heteroconjugate antibodies. In order to generate broadly reactive heteroconjugate antibodies it is useful to select HIV-directed antibodies which recognize a broad range of HIV strains (i.e., antibodies which are not strain specific). It is also useful to select antibodies which are directed against the amino acid sequence: G-P-G-R-A-F; or the amino acid sequence: I-X-I-G-P-G-R, where X is any amino acid; or an epitope within the portion of gp41 from amino acid 584 to amino acid 611. These antibodies can be identified using standard epitope mapping techniques as described below.

Generally, the steps for generating and selecting useful HIV-directed antibodies include: (1) generation of hybridomas and selection of hybridomas producing reactive antibodies; (2) selection of hybridomas producing antibodies capable of binding to cells expressing HIV envelope protein; (3) amplification and purification of selected monoclonal antibodies; (4) analysis of antibody reactivity using gp120 V3 loop peptides or gp41 derived peptides; and (5) epitope mapping. As mentioned above not all of these steps are essential. It is possible to simply follow steps 1 through 3 and use the purified antibodies to prepare heteroconjugate antibodies whose potency and breadth of reactivity can be analyzed using

the reverse transcriptase assay described herein below. To generate a heteroconjugate antibody of the invention, a purified HIV-directed antibody is covalently attached to an antibody directed against an immune effector cell. The potency and reactivity of heteroconjugate antibodies can be measured in a mixed cell culture of effector cells and HIV-infected cells using a reverse transcriptase assay.

Heteroconjugate antibodies may be formed using any convenient cross-linking method. Suitable cross-linking methods include: SPDP, SPDP and SMCC, and biotin-avidin. Segal et al. (U.S. Patent 4,676,980) describes a number of cross-linking techniques. Alternatively, the antibodies can be linked by the generation of bispecific antibodies via hybrid-hybridomas (Suresh et al., *Methods in Enzymology* 121:210, 1896) or by genetic engineering.

Preparation of HIV-directed Antibodies

Antibodies useful for the preparation of the heteroconjugate molecules of the invention may be generated and screened as described below. Methods for preparing and analyzing antibodies directed towards the V3 loop of HIV-MN of an HIV-MN viral variant are also described in U.S. Application No. 07/665,306, filed March 6, 1991, hereby incorporated by reference.

Preparation of the Immunogen

One group of HIV-directed antibodies useful for production of heteroconjugate antibodies recognize sequences within the V3 loop of HIV-MN or an HIV-MN viral variant. Accordingly, the immunogen used to generate these antibodies can include: gp160, gp120, fragments of gp120 or gp160 which include all or part of the V3 loop, or synthetic peptides which include all or part of the V3 loop. In all cases the V3 loop sequences is that of HIV-MN or an HIV-MN viral variant. Preferred immunogens for

generating V3 loop directed antibodies include the RP70 peptide formed into a closed loop (described below).

The other group of HIV-directed antibodies useful for production of heteroconjugate antibodies recognize sequences within the region of gp41 spanning amino acids 584-611. The immunogens used to generate these antibodies can include: gp160, gp41, and fragments of gp160 or gp41 which include all or part of the sequence lying between amino acids 584 and 611 of gp41, i.e., Q-A-R-I-L-A-V-E-R-Y-L-K-D-Q-Q-L-L-G-I-W-G-C-S-G-K-L-I-C.

The immunizing peptide, polypeptide or protein may be in linear form or alternatively may contain the V3 loop formed into a closed loop by creation of a disulfide bond between cysteine residues at the termini of the V3 loop sequence. If the immunizing peptide contains more than one V3 loop, each may be separately formed into a loop through disulfide bonding.

Synthetic peptides containing the desired sequences can be synthesized by automated peptide synthesis using an automated peptide synthesizer. Intact recombinant gp160 envelope polypeptide can be produced in insect cells using a baculovirus expression system and purified as described in Rusche et al., U.S. Application No. 091,481, filed August 31, 1987, assigned to the same assignee as the present invention, hereby incorporated by reference.

Synthetic peptides or protein fragments to be used as immunogens can be either unconjugated or conjugated to an immunogenic carrier, e.g., keyhole limpet hemocyanin (KLH) or ovalbumin, using succinyl maleimidomethyl cyclohexanylcarboxylate (SMCC) as a conjugation agent (Yoshitake et al., *J. Biochem.* 92:1413, 1982), as follows.

Briefly, 1 mg of SMCC dissolved in 50 μl of dimethylformamide is added to 6 mg of carrier (at a

concentration of 10-20 mg/ml in 0.1M $NaPO_4$, pH 6.5) and incubated at room temperature for 0.5 h. The solution is then passed through a Sephadex G-25 column to remove excess unreacted SMCC and 2 mg of peptide is added (suspended in a degassed solution of 0.1M $NaPO_4$, pH 8, 1mM EDTA at a concentration of 10 mg/ml). The solution is mixed by N_2 gas and incubated at 4°C overnight. The sample is then dialyzed in 6M urea, 0.1M $NaPO_4$, pH 7 until the precipitate dissolves. The sample is next eluted through a BioGel P-10 column equilibrated in 6M urea, 0.1M $NaPO_4$. The voided protein is collected and dialyzed in distilled H_2O.

The sequences of several peptides (RP142, RP70, RP342, RP100, RP102, RP108, RP123c, and RP174c) useful in immunogens are shown in Table 1. This list is not meant to be exhaustive; it merely lists a few of the peptides which may be used as immunogens.

Table 1: Examples of Peptides Useful as Immunogens

RP142	Y N K R K R I H I G P G R A F Y T T K N I I G (C)
RP342	I H I G P G R A F Y T
RP70	I N C T R P N Y N K R K R I H I G P G R A F Y T T K N I I G T I R Q A H C N I S
RP100	(S G G) T R K G I H I G P G R A I Y (G G S C)
RP102	(S G G) T R K S I S I G P G R A F (G G S C)
RP108	(S G G) H I G P G R A F Y A T G (G G S C)
RP123c	(C) H I G P G R A F (C)
RP135 (III_B)	N N T R K S I R I Q R G P G R A F V T I G K I G (C)
RP174c	(C) N N T R K S I R I Q R G P G R A F V T I G K I G (C)
RP339 (RF)	I T K G P G R V I Y (C)

Note: Amino acids in parentheses are not in the natural sequence of the indicated isolate

Peptides RP70, RP123c, and RP174c can be formed into closed loops by creation of a disulfide bond between the two cysteine residues near the ends of the amino acid sequence. A method for creating such a bond is described in Zhang et al. (*Biochemistry* 27:3785, 1988).

The peptides were prepared for immunization by emulsification in complete Freund's adjuvant according to standard techniques. (CFA, Difco Labs, Grand Island, NY).

Generation of HIV-Directed Antibodies

HIV-directed antibodies were prepared by intraperitoneal immunization of mouse strains (Balb/c, C57BL/6, A.SW, B10.BR, or B10.A, Jackson Labs., Bar Harbor, ME) with 10-50 μg per mouse of circularized RP70 (Table 1) or recombinant gp160. The mice were given booster immunizations of the immunogen, either in an emulsification of incomplete Freund's adjuvant or in soluble form, two to three times at two to four week intervals following the initial immunization. Mice were bled and the sera assayed for the presence of antibodies reactive with the immunogen. Mice showing a strong serological response were boosted and, 3-5 days later, spleen cells from these mice were fused with NS-1 (A.T.C.C. No. TIB18), SP2-0 (A.T.C.C. No. CRL8287, CRL8006), or P3.X63.AG8.653 myeloma cells incapable of secreting both heavy and light immunoglobulin chains (Kearney et al., *J. Immunol.* 123:1548, 1979) by standard procedures based on the method of Kohler and Milstein, (*Nature* 256:495, 1975).

Supernatants from hybridomas which appeared 6-21 days after fusion were screened for production of antibodies by an ELISA screening assay, as follows. The RP70 peptide was used to screen RP70 generated hybridomas, and a peptide whose amino acid sequence is identical to that of residues 567-647 of gp41, was used to screen gp160 generated hybridomas.

Each well of a 96-well Costar flat-bottom microtiter plate was coated with the peptide by placing a 50 μl aliquot of a PBS solution containing the peptide at a final concentration of 0.1-10 μg/ml in each well. The peptide solution was aspirated and replaced with PBS +

0.5% BSA. Following incubation, the wells were aspirated, washed, and 50 μl of hybridoma supernatant was added. Following incubation, the wells were washed 3 times with PBS, and then incubated with 50 μl of an appropriate dilution of goat anti-mouse immunoglobulin conjugated with horseradish peroxidase (HRP, Zymed Laboratories, San Francisco, CA). The wells were washed again 3 times with PBS and 50 μl of 1mM ABTS (2,2 azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) in 0.1M Na-Citrate, pH 4.2, to which a 1:1000 dilution of 30% H_2O_2 had been added), the substrate for HRP, was added to detect bound antibody. HRP activity was monitored by measuring the absorbance at 410nm.

Hybridomas that test positive by the ELISA method can be tested for their ability to bind to cells which express the HIV envelope protein. In one such assay recombinant vaccinia virus expressing a the *env* gene of a particular HIV strain are used to infect cells of the CD4+ human T-lymphoma line, CEM-ss (AIDS Research and Reference Reagent Program, Rockville, MD, catalog #776). Hybridoma supernatant (or purified antibodies) are incubated with the infected cells, and antibody binding is detected by indirect immune florescence using a secondary antibody and a florescence activated cell sorter. As a control, binding to otherwise identical cells which do not express an HIV *env* gene is measured. Hybridomas producing antibodies which bind to *env* expressing cells (but not to non-expressing cells) are then selected for further characterization. Cells expressing the *env* gene of any HIV strain may be prepared as described below.

In some cases (for neutralizing antibodies) an assay for inhibition of syncytia formation can be used to assess HIV-directed antibodies. In this assay the antibody is added to a mixture of HIV-infected and

uninfected cells and giant cell formation is monitored. This assay is described in detail in U.S. Application No. 07/665,306, filed March 6, 1991, hereby incorporated by reference.

Preparation of HIV *env* Expressing Cells Using a Recombinant Vaccinia Virus

An assay for binding to cells expressing an HIV *env* gene can employ cells infected with a vaccinia virus expressing an HIV *env* gene rather than actual HIV infected cells. Construction of a recombinant vaccinia virus capable of expressing the full-length HIV envelope gene from a vaccinia virus promoter is described in EP Publication No. 0 243 029, hereby incorporated by reference. The recombinant vector pSC25, containing the HIV *env* gene and the *lacZ* gene of *E. coli* expressed from a second vaccinia virus promoter, and flanked by vaccinia viral sequences which together encode thymidine kinase (TK), was used to produce the recombinant virus.

A recombinant vector that contains DNA encoding an envelope gene having the specificity of the HIV-MN variant was prepared by removing a 570 bp *Bgl*II fragment (encoding 180 amino acids) from the HIV-III_B *env* gene which spans the region of the VS loop in pSC25, and replacing it with the analogous *Bgl*II fragment from the HIV-MN *env* gene. The resulting plasmid, pSCR2502, contained a hybrid envelope gene which encoded an envelope protein having the principal neutralizing domain of the MN virus and the remainder of the *env* gene sequence from the HIV-III_B envelope.

A smaller region of the HIV-MN gp160 protein can be used in place of the 180 amino acid replacement just described; e.g., DNA encoding the 36 amino acid V3 loop from any HIV strain can be inserted into the envelope-encoding DNA in place of the corresponding III_B DNA sequence. Alternatively, a recombinant could be used

which contains the complete HIV-MN *env* gene. Multiple HIV envelope expressing strains are useful for assessing the specificity of an antibody.

The recombinant vector pSCR2502 was transfected into CV-1 host cells that had been pre-infected with vaccinia virus containing an intact TK gene. The HIV envelope gene was integrated into the viral DNA by homologous recombination between the TK sequences on the vector and the TK sequences within the viral genome. Recombinants containing the HIV envelope gene were selected by infection of TK- cells and plating on media containing bromodeoxyuridine (BUdR) and X-gal. BUdR is toxic to TK^+ cells and thus selects for TK^- recombinants; X-gal is a chromogenic substrate cleaved by the product of the *lac*Z gene which results in the production of blue plaques where the *lac*Z gene is expressed and further identifies the recombinant virus which also contains the HIV-*env* gene.

Antibody Purification and Amplification

Hybridomas that tested positive for peptide binding in the ELISA assay were subcloned by the limiting dilution method. Hybridoma cells and irradiated splenocytes from nonimmunized syngeneic mice (final concentration 5 cells/ml and 2.5×10^6 cells/ml, respectively) were mixed and 200 μl of the mixed suspension were plated in microtiter wells to give 1 hybridoma cell per well. Subclones which appeared 7-14 days later were assayed again by the ELISA procedure described above. Representative positive subclones were subcloned a second time.

The isotypes of the antibodies were determined by the ELISA method using goat anti-mouse-HRP preparations which corresponded to each of the five major mouse immunoglobulin isotypes (IgM, IgG1, IgG2A, IgG2B and IgG3).

Purified antibodies were prepared by injecting hybridoma subclones that repeatedly tested positive by ELISA and syncytium inhibition assays intraperitoneally into pristane-primed syngeneic mice. The ascites which developed were recovered two to three weeks after injection and the monoclonal antibodies were purified as follows, using procedures which were dependent on the isotype of the antibody. Following elution, all IgG antibodies were dialyzed against PBS.

IgM antibodies were purified by 50% NH_2SO_4 precipitation of ascites fluid from mice injected with the corresponding hybridoma cells, and then dialysis of the precipitate against 4X PBS. The dialyzed antibody was then passed over an Ultrogel A-6 column (Biotechnics, Villeneuve-La-Garenne, France) pre-equilibrated with 4X PBS. The antibody-containing fraction was identified using ELISA.

Ascites fluid containing IgG1 antibodies was diluted 4-fold in 0.1M Tris-HCl, 3M NaCl, pH 8.9, and isolated by passage through a Protein A-Sepharose affinity column equilibrated with the same Tris-NaCl buffer. The antibody was eluted using 0.1M Na-Citrate, pH 6.0.

Ascites fluid containing IgG2 antibodies was diluted two-fold in PBS, and then bound to a Protein-A-Sepharose affinity column equilibrated with PBS. It was then eluted from the column with 0.15M NaCl, 0.1M acetic acid, pH 3.0. Following elution, the antibody was immediately neutralized by the addition of 1M Na_2HCO_3.

Ascites fluid containing IgG3 antibodies was diluted 4-fold in 0.1M Tris-HCl, 3M NaCl, pH 8.9, passed over a Protein-A-Sepharose affinity column, and antibody was eluted from the Protein A column with 0.15M NaCl, 0.1M acetic acid.

Alternatively, all IgG subclasses can be purified by the following procedure. Ascites fluid is diluted 2-fold in 0.1M Tris-HCl, 3M NaCl pH 8.9, passed over Protein A Sepharose affinity column, and eluted with 0.15M NaCl, 0.1M acetic acid, pH 3.0.

Determination of Antibody Specificity

Assays described below can be used for determination of the strain specificity of HIV-directed antibodies and to map the epitope recognized by HIV-directed antibodies. Some or all of these assays may be used to select HIV-directed antibodies for production of heteroconjugate antibodies. The assay for binding to cells expressing an HIV *env* gene described above can also be used to assess antibody specificity. The epitope recognized by the V3-directed antibodies can be mapped using standard ELISA assays and competitive ELISA assays as described below. Peptides which are useful for ELISA assays include: (1) a series of 24 or 25-mers representing the V3 loop sequences from a variety of HIV variants (Table 2); and (2) the MN substitution series, which includes a series of 12-mers corresponding to the MN V3 loop tip sequence (C)-K-R-I-H-I-G-P-G-R-A-F-Y-T-T-(C), each having an alanine residue substituted for one of the amino acids starting at the first arginine (R) residue and proceeding to the tyrosine (Y) residue. In the substitution series a glycine was substituted for the naturally occurring alanine. Antibody recognition of an epitope contained within the MN sequence is revealed by loss of binding of the antibody to an alanine-substituted peptide, the alanine substitution having disrupted the binding interaction.

Competitive ELISA assays were performed as for standard ELISA assays with the following modifications. Prior to applying the antibody to the plate, the antibody preparation is incubated with a test peptide from the

groups listed above at concentrations ranging from 10μM to 0.0045μM. If the test peptide competes with the immobilized immunogen for binding to the antibody, the ELISA will reveal little or no binding of the antibody to the plate.

The epitope recognized by gp41-directed antibodies can be mapped in a similar manner using an alanine substitution series based on the sequence of all or part of gp41. It is also possible to use peptides whose sequence corresponds to a portion of gp41.

V3 Loop-Directed Antibodies

Described below are two antibodies which recognizes sequences within the V3 loop of HIV-MN gp120 and which can be used to generate heteroconjugate antibodies.

Hybridomas F59 and F83 were generated from immunization of BALB/C mice with the closed loop immunogen RP70 (Table 1). Antibodies, designated F59/P5B3 (59.1), and F83/P6F12 (83.1) were identified as antibodies which are not strain specific. Alanine-substituted peptides that were capable of competing with RP70 for binding to the 59.1 antibody did not contain alanine substitutions within the G-P-G-R-A-F sequence. Similarly, peptides that contained the G-P-G-R-A-F sequence were able to compete with RP70 for binding to the 59.1 antibody, while those that did not contain this sequence (i.e., RP129 and RP175) were not able to compete. These results indicate that the 59.1 antibody recognizes the G-P-G-R-A-F epitope. This sequence is present in a wide range of HIV variants. The strain specificity of the 59.1 antibody was analyzed using the above-described techniques. These assays indicated that 59.1 recognizes the V3 loop of HIV-MN, HIV-SF2, HIV-WMJ2 and HIV-III_B.

ELISA assays demonstrated that the 83.1 antibody recognizes the I-X-I-G-P-G-R epitope (where X is any amino acid). The strain specificity of the 83.1 antibody was analyzed using the above-described techniques. These assays indicated that 83.1 recognizes the V3 loop of HIV-MN, HIV-Alabama, HIV-SF2, HIV-WMJ2, and HIV-Duke 7887-7.

Thus, we have identified and characterized two antibodies which recognize a number of HIV strains. One 83.1 recognizes the epitope I-X-I-G-P-G-R (where X is any amino acid. Another, 59.1, recognizes the epitope GPGRAF. THe 59.1 antibody was used to generate a heteroconjugate antibody using the method of Scott et al. (*J. Immunology* 140:8, 1988).

gp41-Directed Antibodies

Recombinant gp160 was used to generate monoclonal antibodies essentially as described above. ELISA assays demonstrated that one of these antibodies, 6C5, recognizes the portion of gp41 from amino acids 584 to 611. This portion of gp41 does not vary significantly from one HIV strain to another. Accordingly antibodies directed against this region are not expected to be strain specific. This antibody was used to generate and purify heteroconjugate antibodies using the method of Scott et al. (*J. Immunology* 140:8, 1988).

OKT3/59.1 and OKT3/6C5 Heteroconjugate Antibodies

The experiments described below illustrate the effect of two heteroconjugate antibodies, OKT3/59.1 and OKT3/6C5, on viral replication in CEM-ss cells (American Type Culture Collection, Bethesda, MD: Accession No. CCL119) infected with various strains of HIV. OKT3/59.1 heteroconjugate antibody was produced by covalently cross-linking an anti-CD3 monoclonal antibody, OKT3, to a second monoclonal antibody, 59.1, directed against an epitope within the V3 domain of the gp120 subunit of HIV-

MN. OKT3/6C5 heteroconjugate antibody was produced by covalently cross-linking OKT3, to a monoclonal antibody, 6C5, directed against a conserved epitope within residues 584-611 of the gp41 subunit of HIV (numbering according to Ratner et al., *Nature* 313:277, 1985). CD3 is a receptor closely associated with the T cell receptor for antigen (TCR). When the infected cells are grown in the presence of cytotoxic T-lymphocytes, which express the CD3 receptor, these heteroconjugate antibodies of the invention dramatically decrease viral replication as measured by viral reverse transcriptase activity. Because reverse transcriptase activity is a sensitive measure of HIV activity, these results indicate that the number of virally infected cells is sharply decreased. Without being bound to a particular theory, it appears that the heteroconjugate antibodies are promoting killing of infected cells by linking T lymphocytes to infected cells.

Generation of CTL

The CTL line (1F8) used for testing the activity of heteroconjugate antibodies was prepared by a modification of the method of Scott et al. (*J. Immunology* 140:8, 1988). Briefly, donor PBL were incubated in bulk culture with an allogenic EBV-transformed lymphoblastoid cell line (stimulator cells) for 7 days in RPMI 1640 medium supplemented with 20% FBS (Gibco/BRL, Grand Island, NY), supernatant derived from PHA-stimulated PBL, and 100 U/ml of recombinant interleukin-2. The cells were then cloned by limiting dilution (1 cell/well) in U-bottom trays. Irradiated autologous PBL and stimulator cells were used as feeders. The clones were screened for CTL activity (assessed by lysis of stimulator cells) and NK activity (lysis of K562 cells; CCL 243, American Type Culture Collection, Bethesda, MD).

Clone 1F8 possessing CTL activity and not NK activity was selected.

OKT3/59.1 Decreases Viral Replication as Measured by Reverse Transcriptase Activity in HIV-MN and HIV-III_B Infected Cells

Unless otherwise noted, CEM-ss cells (15,000 cells/well in a 96 well plate) were infected with 64 infectious units (IU) of HIV-III_B or HIV-MN. At 18 hr post-infection, effector cells (1F8 cells 45,000/well) along with varying concentrations of either OKT3/59.1 heteroconjugate or an equivalent amount of the unconjugated antibodies were added to the infected CEM-ss cells. The CTL were grown in RPMI 1640 with 10% FBS; Gibco/BRL), and were washed with fresh medium prior to addition of antibodies. After 7 days cell-free culture supernatants were harvested and assayed for reverse transcriptase activity by the method of Willey et al. (*J. Virol.* 62:139, 1988).

Referring to Figs. 1 and 2, OKT3/59.1 heteroconjugate (filled diamonds) at 0.5 ng/ml essentially eliminated reverse transcriptase activity in CEM-ss cells infected with either HIV-III_B or HIV-MN. A mixture of unconjugated OKT3 antibody and 59.1 antibody (open squares) had no effect on the reverse transcriptase activity even at 2,000 ng/ml.

Referring to Fig. 3, a separate experiment demonstrated that OKT3/59.1 heteroconjugate has no effect on reverse transcriptase activity in the absence of CTL cells (filled circles). Similarly, CTL in absence of OKT3/59.1 heteroconjugate (filled triangle) have no substantial effect on the reverse transcriptase activity of HIV-III_B infected cells. Unconjugated antibodies in the absence of CTL (open circles) and CTL alone (open triangle) have no substantial effect on the reverse transcriptase activity of infected cells. Uninfected

cells (filled square) have no detectable reverse transcriptase activity. In all cases CTL and/or antibodies were added 18 hr post-infection.

The period of HIV infection prior to the addition of antibody and CTL was varied to determine whether longer periods of viral replication and thus increased viral spread affects the efficacy of the heteroconjugate molecule. CEM-ss cells were incubated with HIV-III_B or HIV-MN (64 IU) for 6, 18, 48 or 72 hours prior to the addition of antibody and CTL. Reverse transcriptase activity was measured 7 days post-infection. When infection proceeded for 6, 18 or 48 hours prior to the addition of OKT3/59.1 heteroconjugate and CTL, 0.5 ng/ml of heteroconjugate was sufficient to completely eliminate reverse transcriptase activity. Under the same conditions, a mixture of unconjugated OKT3 antibody and 59.1 antibody at more than 2,000 ng/ml was required to eliminate reverse transcriptase activity. If infection was allowed to proceed for 72 hours prior to addition of antibody and CTL, 1 ng/ml OKT3/59.1 was required to abolish reverse transcriptase activity. At this time point unconjugated antibody at more than 2,000 ng/ml was required to eliminate reverse transcriptase activity.

The *in vitro* potency of OKT3/59.1 heteroconjugate was further characterized by an experiment in which the concentration of antibody was held constant, but the ratio of CTL to CEM-ss cells was varied. In this experiment the concentration of OKT3/59.1 heteroconjugate (1 μg/ml), the number of CEM-ss cells (15,000/well) and the infectious dose (64 IU) of HIV-III_B or HIV-MN were held constant, and the number of CTL added to the cultures was varied. CTL and/or heteroconjugate antibody were added 18 hr post-infection and reverse transcriptase activity was measured 7 days post infection. Referring to Fig. 4, reverse transcriptase activity was completely

eliminated at a CTL:CEM-ss ratio of 0.1:1 and was partially eliminated ($\geq$ 60%) at CTL:CEM-ss ratios as low as .006:1. This result demonstrates that OKT3/59.1 heteroconjugate is effective even when the number of target cells (CEM-ss) is significantly larger than the number of effector cells, a condition comparable to that observed *in vivo*.

OKT3/59.1 is Effective Against Many HIV Strains

To test whether the OKT3/59.1 heteroconjugate is effective against a variety of HIV strains, CEM-ss cells (150,000/well in 24 well plates) infected with 100-1000 IU of HIV. At the time of infection a 3-fold excess of CTL (450,000/well) and 1 μg/ml of OKT3/59.1 heteroconjugate (or 1 μg/ml of a mixture of monomeric antibodies) were added to the culture. Cultures were split 3 times per week and culture supernatants were collected at four or five day intervals for assay of reverse transcriptase activity. After the initial addition of antibody no further antibody was added. Thus splitting the culture decreases the antibody concentration and the absolute number of target and effector cells. As a control, CEM-ss were cultured with virus only. The HIV isolates tested and their V3 sequences are listed in Table 2.

Table 2: V3 Loop Sequences

Isolate	V3 Sequence
MN	K R K R I H I G P G R A F Y T T K
Alabama	- K S - - - - - - - - - - H - - R
Duke 6587-5	V - N - - - - - - - - - - H - - -
SF2	T - - S - Y - - - - - - - H - - G
WMJ2	V - R S L S - - - - - - - R - R E
III_B	K S I - - Q R - - - - - - V - I G
DUKE 6587-7	T - - G - - - - - - - - I - A - G
DUKE 7887-7	T S R G - R - - - - - - - I L A - E

In this table a "-" indicates that the amino acid at that position is the same as in MN. The conserved GPGRAF motif in underlined

Referring to Fig. 6, compared to CEM-ss without added antibody or CTL (filled circles), OKT3/59.1 (unfilled squares) inhibited reverse transcriptase activity more than 95% in all cases in which the HIV isolate has the GPGRAF sequence (MN, Alabama, Duke 6587-5, III_B, SF2, and WMJ2; panels A, B, D, F, G, and H respectively). Two isolates, Duke 6587-7 (panel E) and Duke 7887-7 (panel C) having a GPGRAI motif were tested, and one (Duke 6587-7) was inhibited. A mixture of unconjugated OKT3 and 59.1 (filled triangles) had no effect.

OKT3/6C5 Heteroconjugate Inhibits Reverse Transcriptase Activity of Infected Cells

OKT3/6C5 heteroconjugate was tested for its ability to inhibit reverse transcriptase activity of HIV-IIIB infected CEM-ss. Briefly, CEM-ss (15,000/well in a 96 well plate) were exposed to 64 IU of HIV-III_B. After 18 hrs., CTL (45,000/well) and OKT3/6C5 heteroconjugate at various concentrations were added. Reverse transcriptase activity was measured 7 days later as described above.

Referring to Fig. 6, OKT3/6C5 heteroconjugate (open circles) essentially eliminated reverse transcriptase activity at concentrations as low as 0.5 ng/ml. In contrast, a mixture unconjugated OKT3 and 6C5 had no significant effect on reverse transcriptase activity.

OKT3/59.1 and OKT3/6C5 Are Cytotoxic in the Presence of CTL

To test the cytotoxic activity of heteroconjugate antibodies, we initially performed ^{51}Cr release assays in a model system using recombinant vaccinia virus-infected CV1 cells. Recombinant vaccinia virus which express either the HIV-III_B *env* gene (VPE16) or HIV-MN (VMN) *env* gene were used to infect CV1 cells. A recombinant vaccinia virus which does not express an HIV *env* gene (VSC8) was used as a negative control. Mixed cell cultures were set up essentially as described for the reverse transcriptase assays. 1F8 cells were used as effector cells and were not by themselves cytotoxic to CV1 cells or vaccinia virus-infected CV1 cells.

Referring to Table 3, OKT3/59.1 heteroconjugate at 10 μg/ml lysed 58% of the VPE16 infected CV1 cells and 62% of the VMN infected CV1 cells. Lyses of uninfected cells was very low. Cell lyses was similarly low when monomeric antibodies were added (data not shown). That the maximum lysis using OKT3/59.1 was 60% as opposed to 100% is probably the result of incomplete infection of CV1 cells by the vaccinia virus (Syncytia formation assays with limiting dilutions of CV1 cells indicated that approximately 50-60% of the cells expressing gp160). Heteroconjugates formed using 1C1 or 7C6, two antibodies which recognize epitopes at the carboxyl-terminus ofgp120 and which bind to cells expressing HIV env (as assessed by FACS), were relatively ineffective in lysing cells infected with vaccinia virus expressing either HIV-MN or

HIV-III$_B$ env protein. Apparently cell surface binding, although necessary, is not a sufficient characteristic by which to determine whether any given antibody can generate a cytotoxic heteroconjugate antibody.

Table 3: Cell Lysis by Heteroconjugate Antibodies

	Percentage Cell Lysis		
Heteroconjugate	CV1-VSC	8CV1-VPE1	CV1-VMN
OKT3/59.1	6	58	62
OKT3/7C6	9	17	N.D.
OKT3/1C1	1	18	N.D.

Assay of Heteroconjugate Potency

The assay described below is used to determine the potency of the heteroconjugate antibodies of the invention. By using a variety of HIV strains the breadth of reactivity of a given heteroconjugate antibody may also be determined. In order to accurately determine the potency of the heteroconjugate antibodies of the invention it is important to measure the effect of the heteroconjugate antibody on HIV infected cells under carefully controlled conditions. The preferred assay is described below.

CEM-ss cells (150,000 cells/well in 24 well microtiter plates, 2 ml wells) are infected with 100-1000 infectious units (IU) of the desired HIV strain. At 18 hr post-infection, 450,000 effector cells are added along with enough heteroconjugate antibody to make the desired initial heteroconjugate antibody concentration. The cells are grown under standard conditions and are split every 3 days. No additional heteroconjugate antibody is added, thus the heteroconjugate antibody

concentration is halved each time the culture is split. A control culture is set up and grown under exactly the same conditions but without heteroconjugate antibody. The reverse transcriptase activity of both cultures is measured 10 days post infection using the method of Willey et al. (*J. Virology* 62:139, 1988). For HIV stains which take longer than 14 days post-infection to reach peak virus production, reverse transcriptase activity should not be measured at 10 days post-infection. Instead the reverse transcriptase activity should be measured at a time which is close to that of maximum virus production.

The infectious units are determined according to the Kärber method. Because viral titer can decrease during storage, it is important that the viral stock be freshly titered. Viral stocks should be carefully prepared so that the number of defective viral particles is low. For example, the multiplicity of infection for preparing viral stocks should be 0.001, cells should be grown under conditions which allow logarithmic cell growth, and virus should be collected at the peak of virus production (as determined by maximal reverse transcriptase activity or p24 expression).

Engineered Heteroconjugate Antibodies

Since, for the most part, monoclonal antibodies are produced in species other than humans, they are often immunogenic to humans. In order to successfully use heteroconjugate antibodies in the treatment of humans, it may be necessary to create chimeric antibody molecules wherein the antigen binding portion (the variable region) is derived from one species, and the portion involved with providing structural stability and other biological functions (the constant region) is derived from a human antibody. Methods for producing chimeric antibodies in which the variable domain is derived from one species and

the constant domain is derived from a second species are well known to those skilled in the art. See, for example, Neuberger et al., WO Publication No. 86/01533, priority September 3, 1984; Morrison et al, EP Publication No. 0,173,494, priority August 27, 1984. An alternative method, in which an antibody is produced by replacing only the complementarity determining regions (CDRs) of the variable region with the CDRs from an immunoglobulin of the desired antigenic specificity, is described by Winter (GB Publication No. 2,188,638, priority March 27, 1986). Murine monoclonals can be made compatible with human therapeutic use by producing an antibody containing a human Fc portion (Morrison, *Science* 229:1202, 1985). Single polypeptide chain antibodies are also more easily produced by recombinant means than are conventional antibodies. Ladner et al. (U.S. Patent No. 4,946,778) describes methods for producing single polypeptide chain antibodies and these methods may be adapted to produce heteroconjugate antibodies. Established procedures would allow construction, expression, and purification of such a hybrid monoclonal antibody. Quadromas can be used to generate bispecific antibodies (Reading et al., U.S Patent Nos. 4,474,893 and 4,714,681).

Use

The antibody of the invention is administered parenterally, either via the intravenous or intramuscular route. A typical treatment regimen would comprise administration of an effective amount of antibody administered over between about one week and about 6 months. The number of treatments required to control a patient's disease may vary from individual to individual, depending upon the severity and stage of the illness and the individual characteristics of each patient being treated. The total dose required for each treatment may

be administered by multiple doses or in a single dose. The human monoclonal antibody may be administered alone or in conjunction with other HIV treatments, such as AZT, in order to control a patient's disease.

Pharmaceutical compositions of heteroconjugate antibodies are produced according to the intended mode of administration and may include: liposomes, solutions, suspensions and microparticles.

In some circumstances it may be desirable to administer the heteroconjugate antibody along with the appropriate effector cell (Nitta et al., *The Lancet* 335:368, 1990). For example, peripheral blood lymphocytes (PBL) may be collected from an individual in need of treatment for HIV infection (or a compatible donor) and incubated with a heteroconjugate antibody prior to reinfusion of the cells. In some cases the PBL may be expanded in culture (Rosenberg et al., *Science* 233:1318, 1986). The PBL may also be incubated with interleukins, interferons, or other immunomodulators. In addition the cells may be incubated with molecules such as receptor specific antibodies which will stimulate the cytolytic activity of the effector cells (Scott et al., *Cellular Immunology* 114:370, 1988).

What is claimed is:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Repligen Corporation

(ii) TITLE OF INVENTION: HETEROCONJUGATE ANTIBODIES FOR TREATMENT OF HIV INFECTION

(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:	Fish & Richardson
(B) STREET:	225 Franklin Street
(C) CITY:	Boston
(D) STATE:	Massachusetts
(E) COUNTRY:	U.S.A.
(F) ZIP:	02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:	3.5" Diskette, 1.44 Mb
(B) COMPUTER:	IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM:	IBM P.C. DOS (Version 3.30)
(D) SOFTWARE:	WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:	
(B) FILING DATE:	
(C) CLASSIFICATION:	

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:	07/699,773
(B) FILING DATE:	14-May-1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Paul T. Clark
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00231/055WO1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Gly Pro Gly Arg Ala Phe
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ile Xaa Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu
5 10 15

Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
5 10 15

Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr
5 10 15
Thr Thr Lys Asn Ile Ile Gly Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Asn Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile
5 10 15
Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys Asn Ile Ile Gly Thr Ile
20 25 30
Arg Gln Ala His Cys Asn Ile Ser
35 40

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ser Gly Gly Thr Arg Lys Gly Ile His Ile Gly Pro Gly Arg Ala Ile
5 10 15
Tyr Gly Gly Ser Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ser Gly Gly Thr Arg Lys Ser Ile Ser Ile Gly Pro Gly Arg Ala Phe
5 10 15
Gly Gly Ser Cys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ser Gly Gly His Ile Gly Pro Gly Arg Ala Phe Tyr Ala Thr Gly Gly
5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Cys His Ile Gly Pro Gly Arg Ala Phe Cys
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala
5 10 15

Phe Val Thr Ile Gly Lys Ile Gly Cys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Cys Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg
5 10 15
Ala Phe Val Thr Ile Gly Lys Ile Gly Cys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr Cys
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Cys Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Cys
5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
5 10 15
Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Lys Lys Ser Arg Ile His Ile Gly Pro Gly Arg Ala Phe His Thr Thr
5 10 15
Arg

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Val Arg Asn Arg Ile His Ile Gly Pro Gly Arg Ala Phe His Thr Thr
5 10 15
Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Thr Arg Lys Ser Ile Tyr Ile Gly Pro Gly Arg Ala Phe His Thr Thr
5 10 15
Gly

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Val Arg Arg Ser Lys Ser Ile Gly Pro Gly Arg Ala Phe Arg Thr Arg
5 10 15
Glu

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
5 10 15
Gly

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Thr Arg Lys Gly Ile His Ile Gly Pro Gly Arg Ala Ile Tyr Ala Thr
5 10 15
Gly

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Thr Ser Arg Gly Ile Arg Ile Gly Pro Gly Arg Ala Ile Leu Ala Thr
5 10 15
Glu

Claims

1. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein said heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

2. The heteroconjugate antibody of claim 1 where in said decrease in said reverse transcriptase activity of said first cell culture is greater than 90% compared to said reverse transcriptase activity of said second mixed cell culture.

3. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an

antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein said heteroconjugate antibody at an initial concentration of 10 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

4. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein said heteroconjugate antibody at an initial concentration of 5 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared

to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

5. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against a V3 loop sequence of the gp120 envelope protein of HIV MN or a HIV MN viral variant expressed on the surface of HIV-infected cells, wherein said heteroconjugate antibody at an initial concentration of 1 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection,

and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

6. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against the amino acid sequence GPGRAF.

7. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against the amino acid sequence IXIGPGR, wherein X = any amino acid.

8. The heteroconjugate antibody of claim 6 or claim 7 wherein said heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after

infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

9. The heteroconjugate antibody of claim 1 wherein said heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-III_B decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-III_B, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-III_B.

10. The heteroconjugate antibody of claim 1 wherein said heteroconjugate antibody at an initial concentration of 20 ng/ml in three or more mixed cell cultures each of which comprises said effector cells and CEM-ss cells infected with one of the HIV strains: Alabama, Duke 6587-5, Duke 6587-7, Duke 7887-7, SF2, WMJ2, and IIIB, decreases the reverse transcriptase activity of each of said mixed cell culture by 80% compared to the reverse transcriptase activity of an otherwise identical mixed cell culture comprising said effector cells and CEM-ss cells infected with said same strain of HIV, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second

mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of said strain of HIV.

11. A heteroconjugate antibody comprising a first and a second portion joined together covalently, said first portion comprising an antibody directed against an antigen present on the surface of an effector cell of the peripheral blood, said second antibody portion comprising an antibody directed against the amino acid sequence QARILAVERYLKDQQLLGIWGCSGKLIC.

12. The heteroconjugate antibody of claim 11 wherein said heteroconjugate antibody at an initial concentration of 20 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 80% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of HIV-MN.

13. The heteroconjugate of claim 1 or claim 11 wherein said effector cell is chosen from the group consisting of cytotoxic T lymphocytes, neutrophils, monocytes/macrophages, and large granular lymphocytes.

14. The heteroconjugate of claim 1 or claim 11 wherein said antigen present on the surface of an effector cell is CD3.

15. The heteroconjugate antibody of claim 1 wherein said heteroconjugate antibody at an initial concentration of 200 ng/ml in a first mixed cell culture comprising said effector cells and CEM-ss cells infected with an HIV strain other than HIV-MN decreases the reverse transcriptase activity of said first mixed culture cell by at least 50% compared to the reverse transcriptase activity of an otherwise identical second mixed cell culture comprising said effector cells and said CEM-ss cells infected with said HIV strain other than HIV-MN, wherein said effector cells are in 3-fold excess over said CEM-ss cells in said first and second mixed cell cultures, said reverse transcriptase activity is measured ten days after infection, said heteroconjugate antibody and said effector cells are added to said CEM-ss cells in said first mixed cell culture 18 hours after infection, and said first and second cell cultures are infected with 100-1000 infectious units of said HIV strain other than HIV-MN.

16. The heteroconjugate antibody of claim 1 wherein said heteroconjugate antibody binds to the V3 loop of an HIV strain other than HIV-MN.

17. A pharmaceutically acceptable composition comprising a pharmaceutically effective amount of a heteroconjugate antibody of claim 1 or claim 11.

18. An HIV-targeted effector cell comprising:

(a) an effector cell expressing a cell surface antigen; and

(b) the heteroconjugate antibody of claim 1 or claim 11.

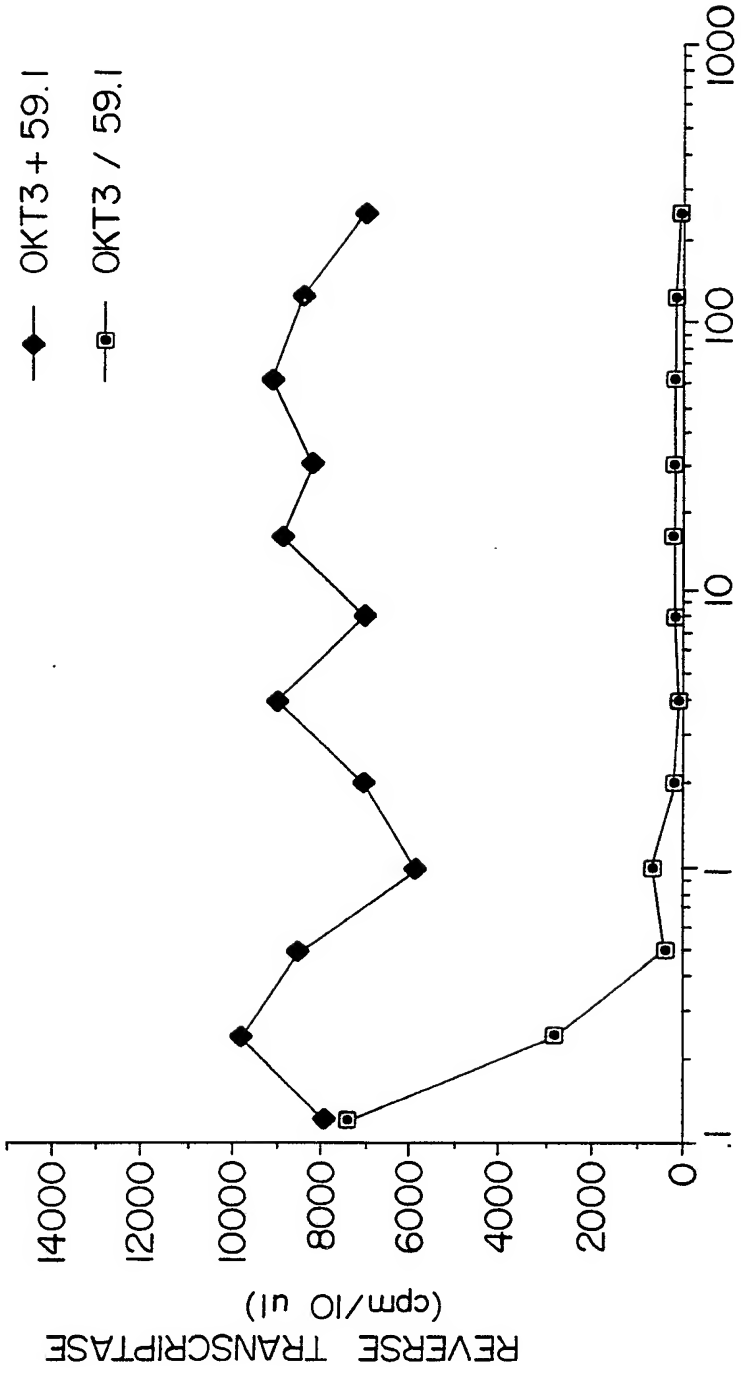
OKT3 + 59.1
OKT3 / 59.1
REVERSE TRANSCRIPTASE
(cpm/10 ul)
14000
12000
10000
8000
6000
4000
2000
0
.1
1
10
100
1000
MAb ng/ml

FIG. 1

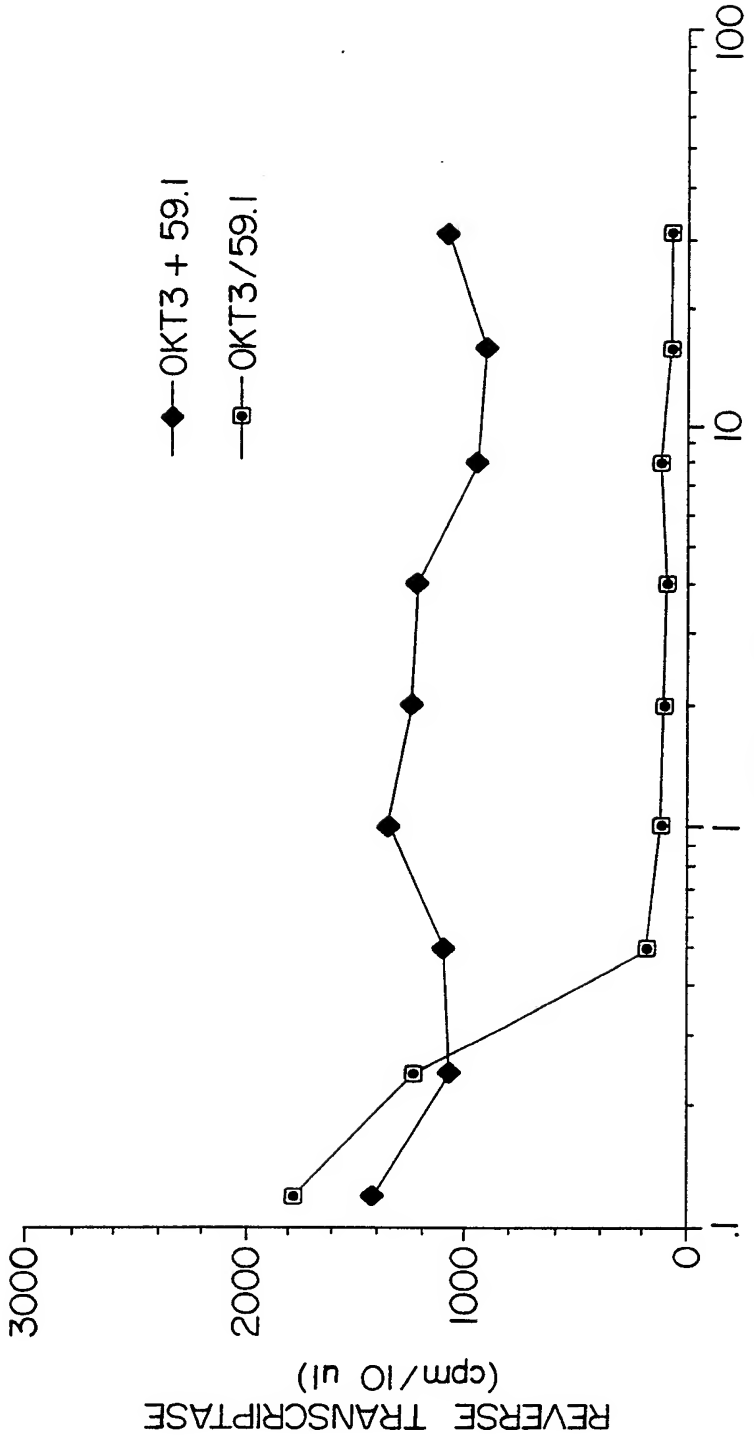
OKT3 + 59.1
OKT3/59.1
REVERSE TRANSCRIPTASE
(cpm/10 ul)
3000
2000
1000
0
.1
1
10
100
MAb ng/ml

FIG. 2

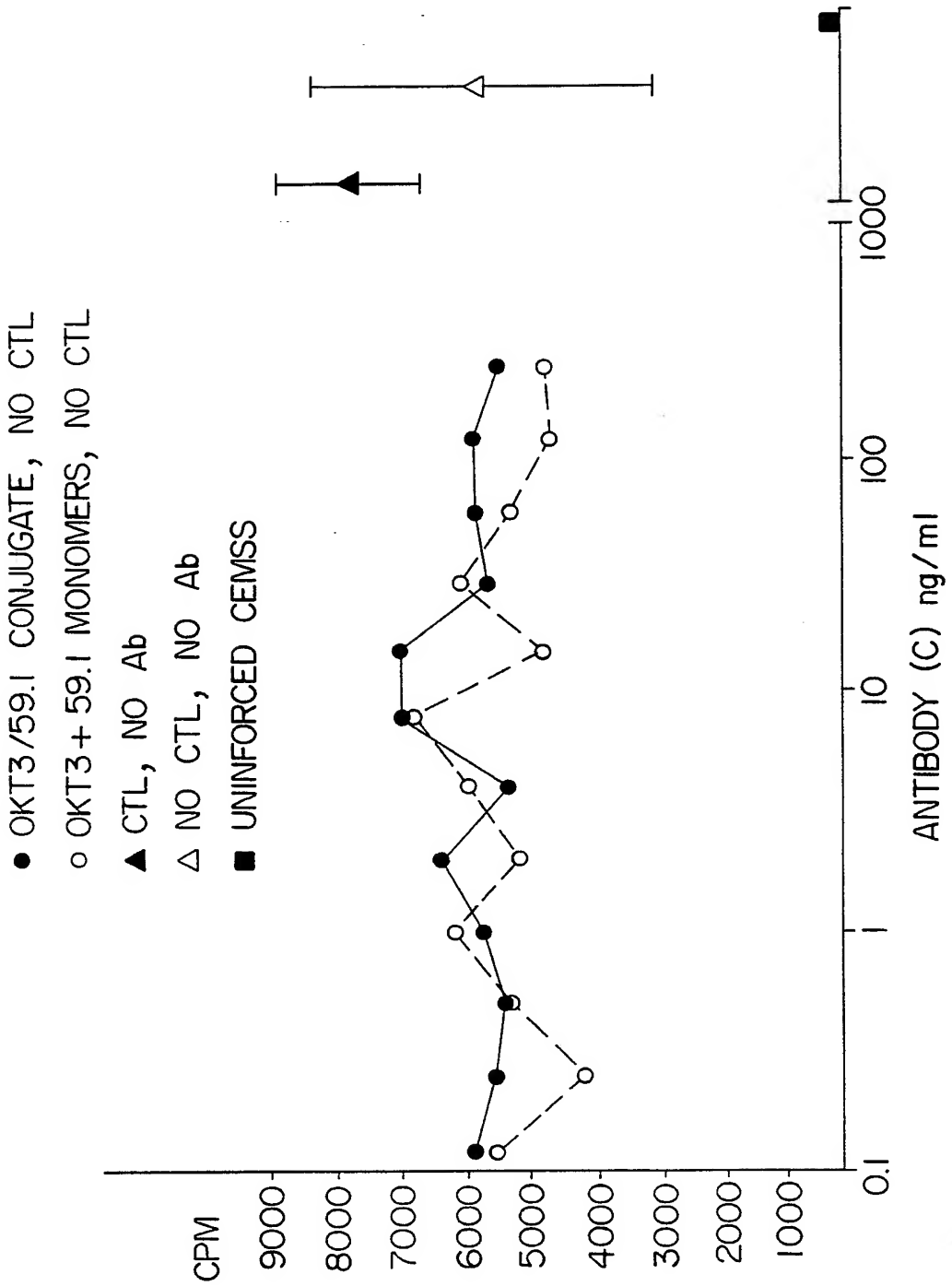
● OKT3/59.1 CONJUGATE, NO CTL
○ OKT3 + 59.1 MONOMERS, NO CTL
▲ CTL, NO Ab
△ NO CTL, NO Ab
■ UNINFORCED CEMSS
CPM
9000
8000
7000
6000
5000
4000
3000
2000
1000
0.1
1
10
100
1000
ANTIBODY (C) ng/ml

FIG. 3

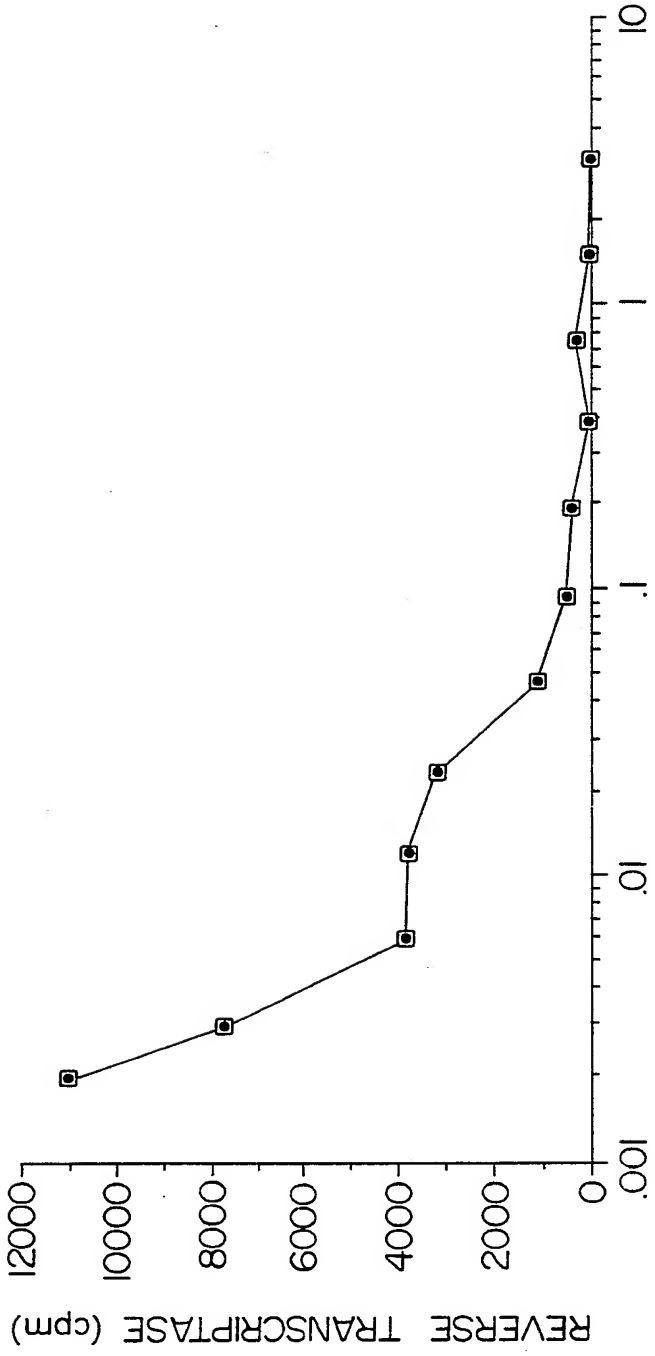
REVERSE TRANSCRIPTASE (cpm)
12000
10000
8000
6000
4000
2000
0
.001
.01
.1
1
10
E:T RATIO

FIG. 4

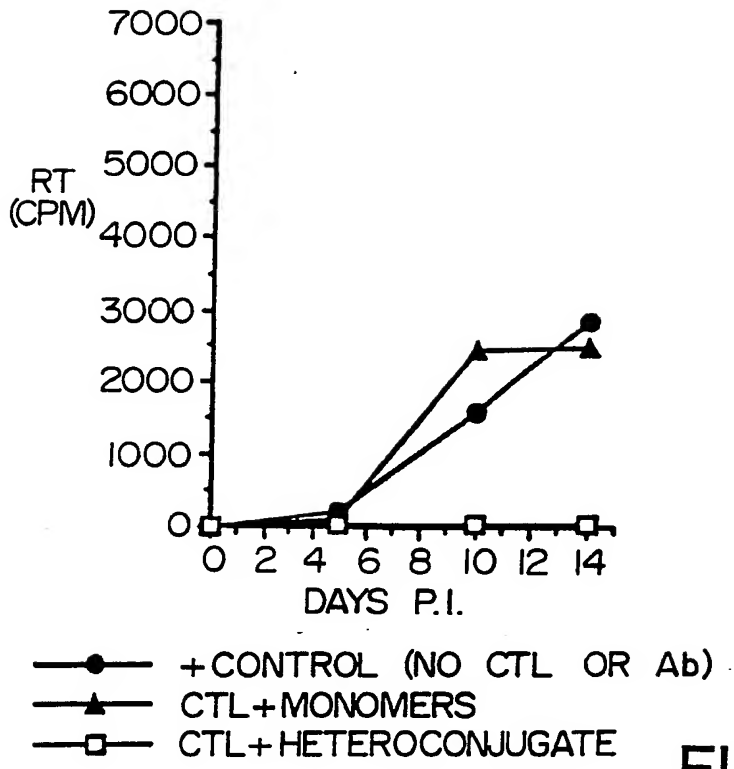
7000
6000
5000
4000
3000
2000
1000
0
RT (CPM)
0 2 4 6 8 10 12 14
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL+HETEROCONJUGATE

FIG. 5(A)

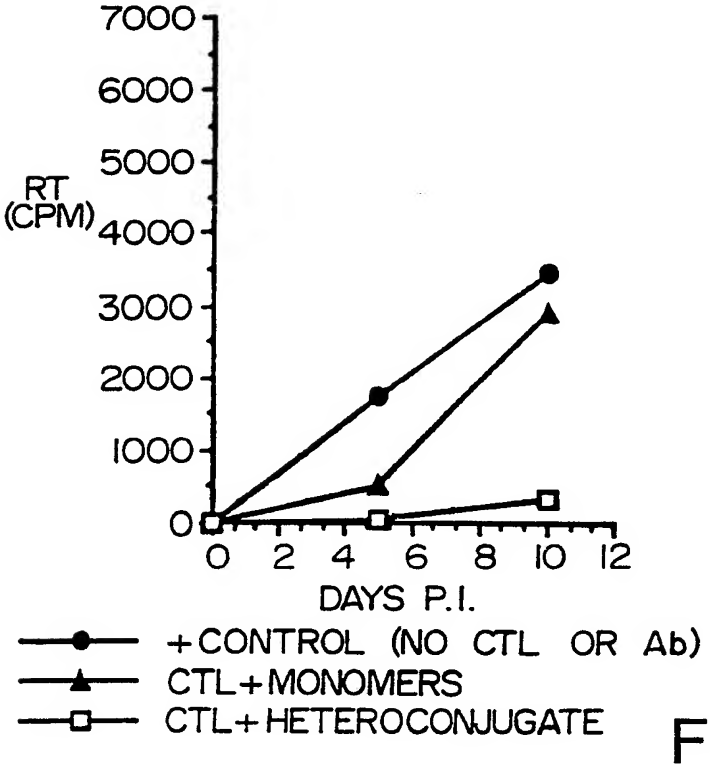
7000
6000
5000
4000
3000
2000
1000
0
RT (CPM)
0 2 4 6 8 10 12
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL+HETEROCONJUGATE

FIG. 5(B)

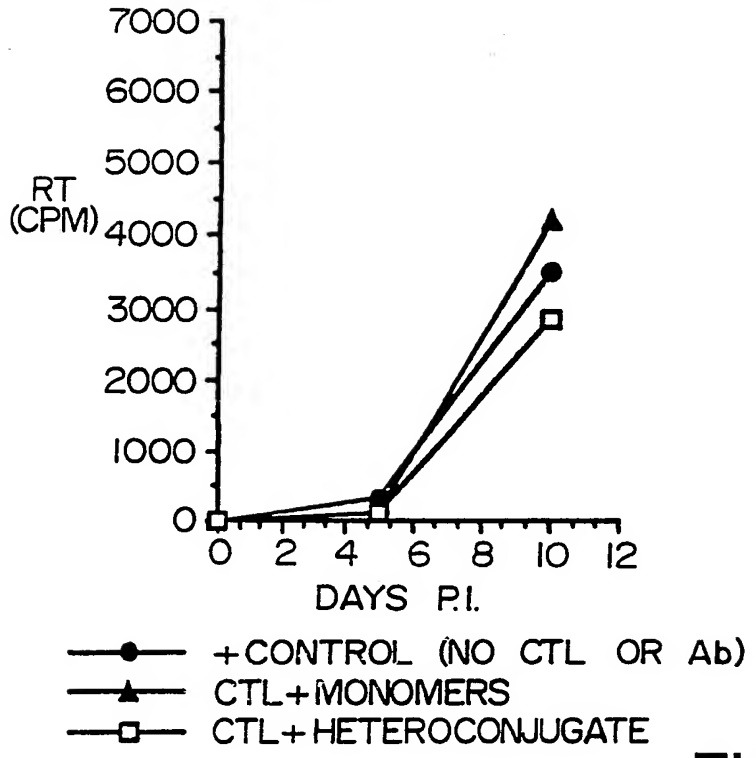
7000
6000
5000
RT
(CPM)
4000
3000
2000
1000
0
0 2 4 6 8 10 12
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL+HETEROCONJUGATE

FIG. 5(C)

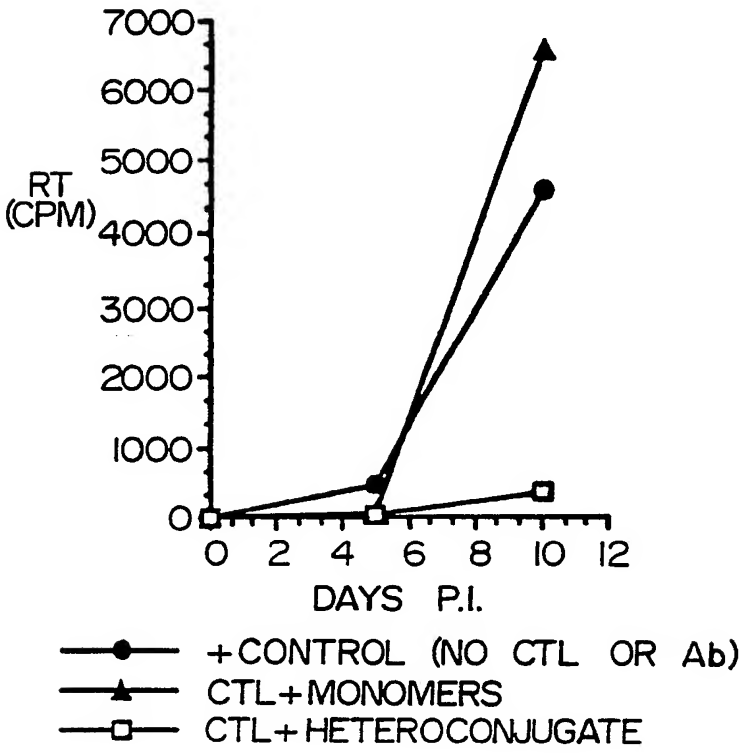
7000
6000
5000
RT
(CPM)
4000
3000
2000
1000
0
0 2 4 6 8 10 12
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL+HETEROCONJUGATE

FIG. 5(D)

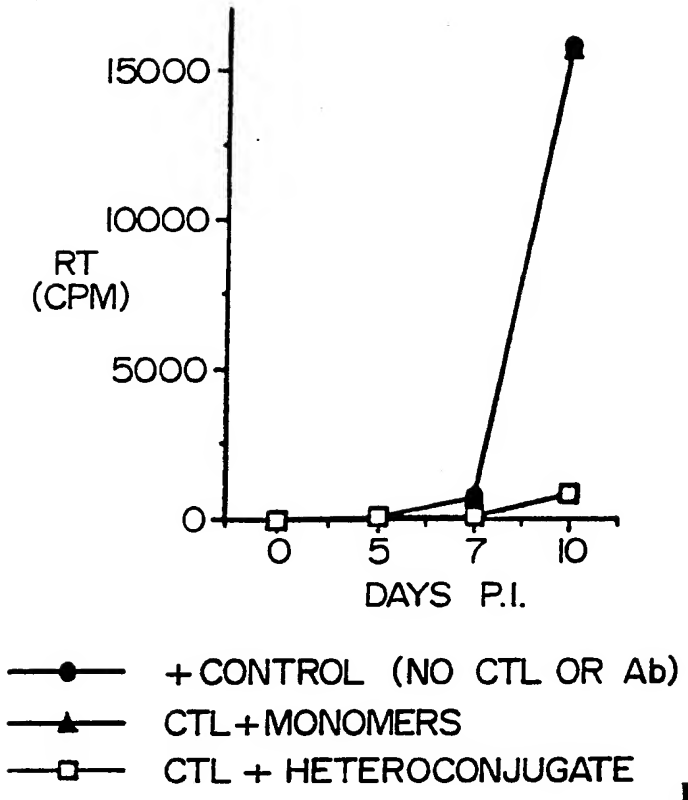
15000
10000
RT
(CPM)
5000
0
0
5
7
10
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL + HETEROCONJUGATE

FIG. 5(E)

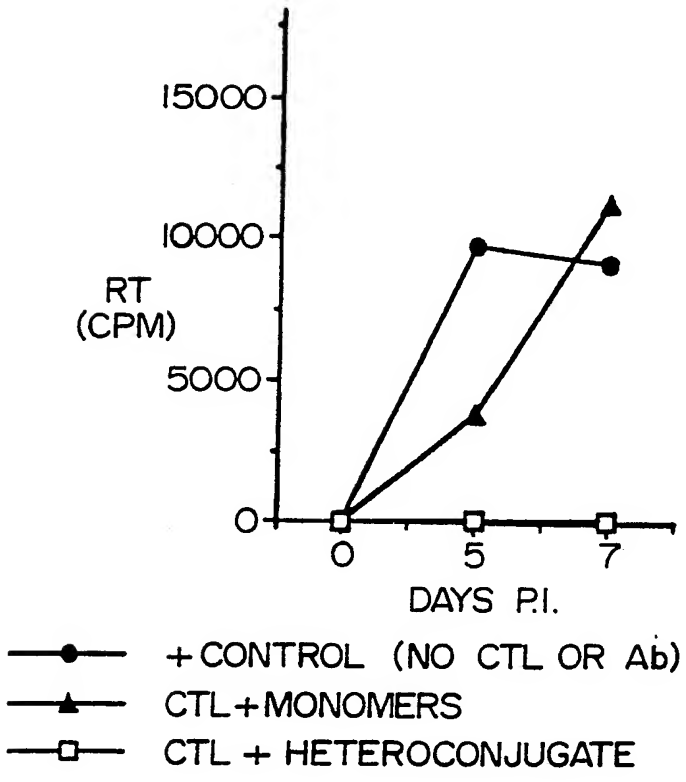
15000
10000
RT
(CPM)
5000
0
0
5
7
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL + HETEROCONJUGATE

FIG. 5(F)

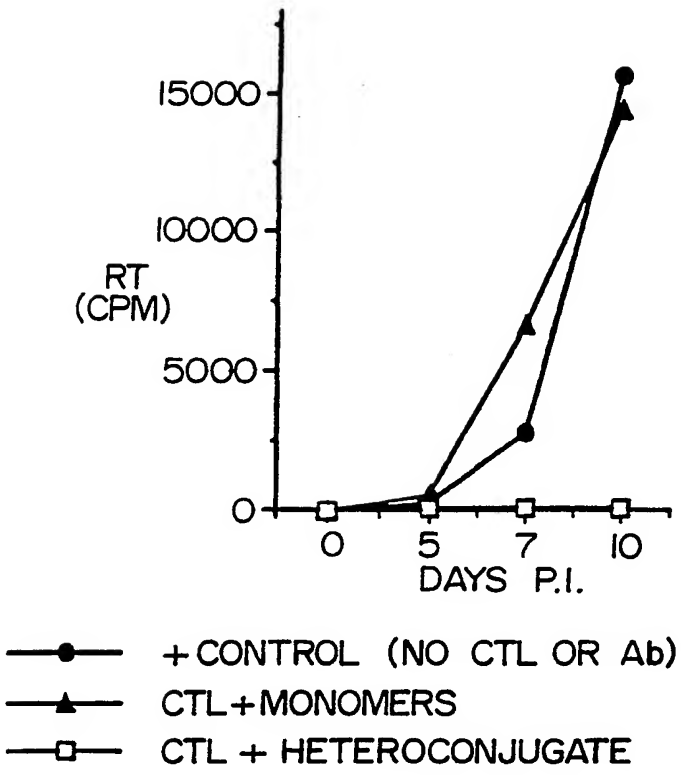
15000
10000
RT
(CPM)
5000
0
0
5
7
10
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL + HETEROCONJUGATE

FIG. 5(G)

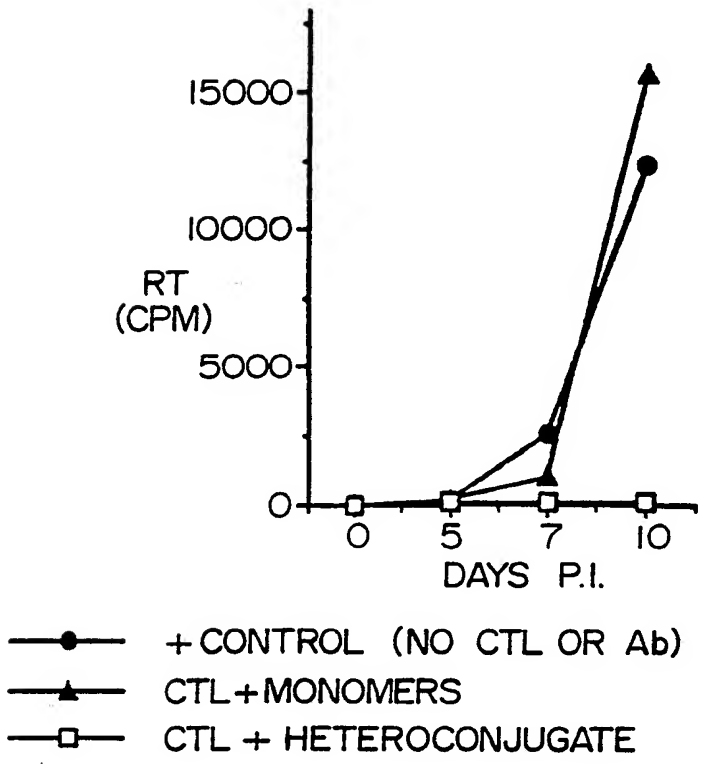
15000
10000
RT
(CPM)
5000
0
0
5
7
10
DAYS P.I.
+CONTROL (NO CTL OR Ab)
CTL+MONOMERS
CTL + HETEROCONJUGATE

FIG. 5(H)

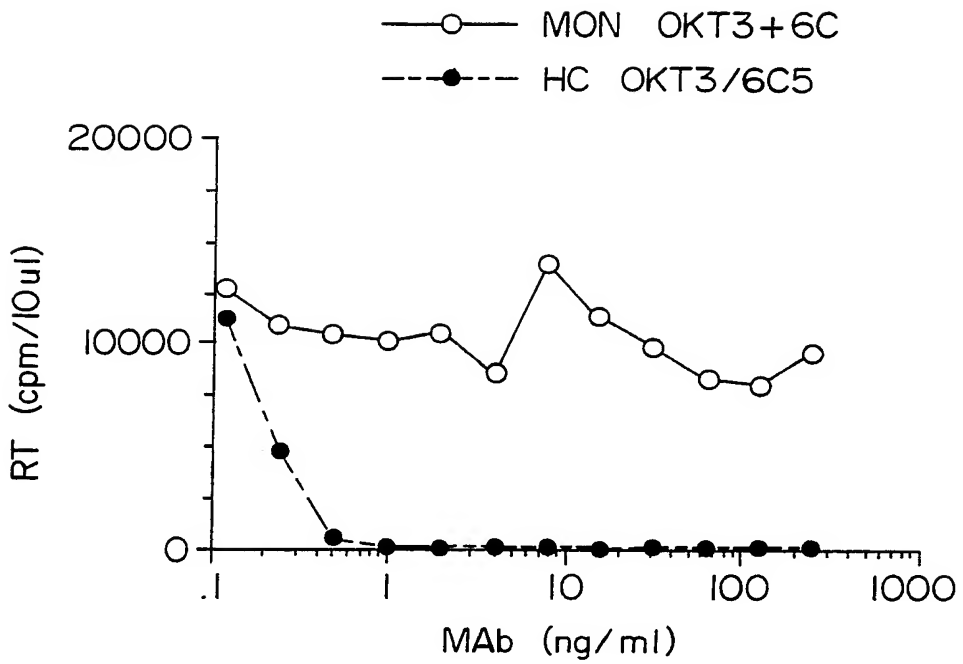
MON OKT3+6C
HC OKT3/6C5
20000
10000
0
RT (cpm/10ul)
.1
1
10
100
1000
MAb (ng/ml)

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03616

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

AUTOMATED PATENT SYSTEM, FILE: USPAT; DIALOG, FILE:BIOSIS, CAS, MEDLINE, PASCAL, WPI, EMBASE
SEARCH TERMS:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOTECHNOLOGY, VOLUME 3, ISSUED OCTOBER 1985, CHANG ET AL, "DETECTION OF ANTIBODIES TO HUMAN T-CELL LYMPHOTROPIC VIRUS-III (HTLV-III) WITH AN IMMUNOASSAY EMPLOYING A RECONIANNAT ESCHERICIA COLI-DERIVED VIRAL ANTIGENIC PEPTIDE", PAGES 905-909, ENTIRE DOCUMENT	1-6, 8-10, AND 13-17
Y	VIRILOGY, VOLUME 164, ISSUED 1988, GURGO ET AL, "ENVELOPE SEQUENCES OF TWO NEW UNITED STATES HIV-1 ISOLATES", PAGES 531-536, ENTIRE DOCUMENT.	1-6, 8-10, AND 13-17
Y	JOURNAL OF VIROLOGY, VOLUME 61 NO. 2, ISSUED FEBRUARY 1987, MODROW ET AL, "COMPUTER ASSISTED ANALYSIS OF ENVELOPE PROTEIN SEQUENCES OF SEVEN HUMAN IMMUNODEFICIENCY VIRUS ISOLATES: PREDICTION OF ANTIGENIC EPTIOPES IN CONSERVED AND VARIABLE REGIONS", PAGES 570-578, ENTIRE DOCUMENT.	1-6, 8-10, AND 13-17
Y	WO, A, 91/00360 (FANGER ET AL), 10 JANUARY 1991, ENTIRE DOCUMENT.	1-6, 8-10, AND 13-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 23 June 1992	Date of mailing of the international search report 30 SEP 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer T. MICHAEL NISBET Telephone No. (703) 308-4204

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03616

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 39/00, 39/42, 35/14; C12Q 1/00; G01N 33/53; C12P 21/02; C12N 9/96, 5/00, 15/00; C07K 3/00, 13/00, 15/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/85.8, 86; 435/7.1, 70.21, 188.5, 240.27, 965, 972, 974; 436/819, 822; 530/387.3, 387.9, 388.3, 388.35, 388.7, 388.75, 389.4, 867; 930/221; 935/15, 93, 101, 105

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/85.8, 86; 435/7.1, 70.21, 188.5, 240.27, 965, 972, 974; 436/819, 822; 530/387.3, 387.9, 388.3, 388.35, 388.7, 388.75, 389.4, 867; 930/221; 935/15, 93, 101, 105

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. CLAIMS 1-6,8-10, AND 13-17 CONTAIN CLAIMS TO SEPARATE ANTIBODY CONJUGATES WHICH ARE FACILITATE THE TREATMENT OF AIDS INFECTION.

II. CLAIM 7 IS DRAWN TO A SPECIAL ANTIBODY CONJUGATE WHICH BINDS A SEPARATE SEQUENCE FROM THE ONE DESCRIBED ABOVE IN GROUP I. AS SUCH THE DIFFERENT BINDING SEQUENCE REPRESENTS A SEPARATE PRODUCT.

III. CLAIMS 11-12 ALSO DEAL WITH AN ANTIBODY CONJUGATE WHICH BINDS A SEPARATE SEQUENCE FROM EITHER THE TWO ANTIBODY CONJUGATES OF GROUPS I OR II. THEREFORE, THIS TECHNICALLY REPRESENTS A MATERIALLY SEPARATE PRODUCT.

IV. CLAIM 18 CLAIMS A MATERIALLY SEPARATE COMPOSITION OF CELLS CONTAINING THE ANTIBODY CONJUGATES OF THE PREVIOUS CLAIMS. THIS IS A SEPARATE PRODUCT AND IS PROPERLY RESTRICTABLE.